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(54) **Normal human growth regulatory receptor for TGF beta.**

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in their precursor structures. TGF-β3, the amino acid sequence of which has very recently been deduced from cDNA clones, appears to contain a C-terminal 112 amino acid sequence with about 80% homology to the mature monomers of TGF-β1 and TGF-β2 (Dijke et al., 1988, Proc. Natl. Acad. Sci. USA 85:4715-4719). TGF-β1.2 is a heterodimeric form comprising a β1 and β2 subunit linked by disulfide bonds (Cheifetz et al., 1987, Cell 48:409-415).

2.2. TGF-β RECEPTORS

Three types of TGF-β receptors have been described and defined as types I, II and III on the basis of individual structural and functional properties. The type III receptor for TGF-β is a large, disulfied-linked glycoprotein containing a 280-330 kilodalton subunit within which the ligand-binding site is located (Massague, 1985, J. Biol. Chem. 260:7059-7066). Although TGF-β1 and TGF-β2 bind differentially to the type I and type II receptors, the type III receptor exhibits similar affinity for both ligands (Cheifetz et al., 1987, Cell 48:409-415).

Occupancy of type III receptors has been implicated in the mediation of several cellular responses to both TGF-β1 and TGF-β2 including the stimulation of fibronectin (Ignotz and Massague, 1986, J. Biol. Chem. 61:4337-4345), type I collagen (Roberts et al., 1986, Proc. Natl. Acad. Sci. USA 83:4167-4171), cell adhesion receptor synthesis and expression (Ignotz and Massague, 1987, Cell 51:189-197) and chondroitin/dermatan sulfate proteoglycans (Bassols and Massague, 1988, J. Biol. Chem. 263:3039-3045). The type III receptor may play a pivotal role in mediating changes in the extracellular matrix and cell adhesion properties induced by TGF-β, resulting in the induction or repression of certain cell phenotypes and cell proliferation, i.e., induced proliferation of fibroblasts in semi-solid medium (Assoian et al., 1983, J. Biol. Chem. 258:7155-7160); inhibition of the proliferation of epithelial cells (Roberts et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123), T and B lymphocytes (Kehrl et al., 1986, J. Exp. Med. 163:1037-1050; Kehrl et al., 1985, Clin. Res. 33:610-615), thymocytes (Ristow, 1986, Proc. Natl. Acad. Sci. USA 83:5531-5533), and certain tumor cells (Roberts et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123); stimulation of chondrogenesis (Seyedin et al., 1985, Proc. Natl. Acad. Sci. USA 82:2267-2271), osteogenesis (Centrella et al., 1986, Endocrinology 19:2306-2312) and epithelial cell differentiation (Masui et al., 1986, Proc. Natl. Acad. Sci. USA 83:2438-2442); and inhibition of expression of adipose (Ignotz et al., 1985, Proc. Natl. Acad. Sci. USA 82:8530-8534), skeletal muscle (Olson et al., 1986, J. Cell. Biol. 103:1799-1805) and hematopoietic (Ohta et al., 1987, Nature 329:539-541) phenotypes. High affinity receptors for TGF-β have been found on nearly all cells examined to date, including cells of epithelial, mesenchymal, and hematopoeitic origin, on both normal and tumor cells, cells of adult or embryonic origin, and cells from different species (Wakefield et al., 1987, J. Cell. Biol. 105:965).

Biologically latent TGF-β is unable to bind to its receptor or generate a biological response, indicating that activation of TGF-β is required before the TGF-β receptor will recognize and bind its ligand (Wakefield et al., 1987, J. Cell Biol. 105:965). Neither the mechanisms behind TGF-β activation nor the reasons for the inability of TGF-β receptor to recognize and bind latent TGF-β are understood.

Results from a variety of studies involving the TGF-βs and their receptors have raised interesting questions regarding the role of the TGF-β system in tumorigenesis. Although TGF-β receptors appear to be universally expressed on all cell types, some notable exceptions have been found; for example, most retinoblastoma cell lines appear not to have functional TGF-β receptors, the absence of which may allow these cells to escape the otherwise normal growth-controlling actions of TGF-β (Kimichi et al., 1988, Science 240:196-199). In this regard, the expression or loss of expression of factors which regulate the synthesis and/or expression of the TGF-β receptor may lead to a concomitant loss of responsiveness to the growth restraining effect of TGF-β. Another mechanism by which tumor cells may lose their ability to respond to and be regulated by TGF-β may involve a cells inability to activate the latent form of TGF-β, thereby blocking the growth inhibitory signal generated by ligand-receptor binding. (Wakefield, 1987, J. Cell. Biol. 105:965-975).

A role for TGF-β and its receptor in early mammalian development has been suggested. Critical structures involved in the morphogenesis of early mouse embryos have shown specific immunohistochemical localization of TGF-β (Heine et al., 1987, Proc. Am. Assoc. Cancer Res. 28:53). TGF-β exhibits either a growth promoting or growth inhibiting effect on cultured normal fetal fibroblasts, depending on the gestational age of the fetus of origin, suggesting that a maturational change in the biology of TGF-β may occur between 13 and 16 weeks into human gestation (Hill et al., 1986, 128:322-328). In addition, murine embryonal carcinoma cells which appear to have few, if any, receptors for TGF-β, express receptors and

in the absence of 2-mercaptoethanol. Coomassie staining revealed no detectable amounts of TGF-β in lane 2 indicating a coupling efficiency of better than 90%.

FIG. 9 Mobility of purified TGF-β receptor on 6.25% SDS-PAGE (5% stacking gel) under reducing conditions. Only one Coomassie-stainable band is visible (migrating close to the myosin marker) at a calculated molecular mass of 200-206 Kd.

FIG. 10 Ability of purified TGF-β receptor to bind TGF-β. Affinity purified receptor preparations were crosslinked to bound [^{125}I]-iodo-TGF-β with DSS and analyzed on SDS-PAGE. Lanes marked (-) contained only [^{125}I]-iodo-TGF-β; lanes marked (+) contained [^{125}I]-iodo-TGF-β in the presence of 1,000 ng/ml unlabeled TGF-β. The 240 Kd band is specifically labeled with radioligand and has the same mobility on SDS-PAGE as the receptor-TGF-β complex from cell surface membranes (see FIG. 7).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the production of a purified receptor for transforming growth factor-beta (TGF-β). The purified TGF-β receptor of the invention, a type III TGF-β receptor, may be isolated from cells which naturally express such receptors on their surfaces. Alternatively, cells which have been transfected with expression vectors containing the type III TGF-β receptor gene and which are capable of directing the synthesis of the mature, active receptor may be used as a source for isolating TGF-β receptor.

The method of the invention is demonstrated herein, by way of examples, in which the type III TGF-β receptor was identified and purified from HEPM cells, characterized, and used to obtain compositional and structural information. HEPM cells were found to express excessively high levels of type III TGF-β receptors, making these cells an excellent source for the isolation and purification of this receptor. In a specific embodiment, type III TGF-β receptor was purified to homogeneity by affinity chromatography. Specifically, recombinant TGF-β1 was immobilized onto polymeric resin and used to isolate type III TGF-β receptor from a solution containing solubilized HEPM cell membranes. The affinity purified type III TGF-β receptor isolated from HEPM cells has a molecular mass of about 200-206 kd and is capable of specifically binding TGF-β1 and TGF-β2. The amino acid composition and partial amino acid sequence of the type III TGF-β1 receptor was determined.

Purified type III TGF-β receptor may find use in the treatment of a wide variety of cell-growth related disorders and, generally, as a means for controlling cell growth and differentiation. For example, purified type III TGF-β receptor may be useful as a therapeutic scavenging agent in situations where constraining the activity of TGF-β is desirable. In this regard, the use of the receptor as a scavenging agent could effectively inhibit binding of TGF-β to any of its natural target receptors, thereby blocking the cell proliferative or inhibitory signals generated by the ligand-receptor binding event. Since it appears that the vast majority of cell types are susceptible to TGF-β action, it may be useful to incorporate type III TGF-β receptors in drug delivery systems designed to localize the scavenging effect of the receptor to a certain population of cells, cell types, or specific tissues or organs. Antibodies to which the type III TGF-β receptors have been conjugated may be used to target scavenging activity to particular cells or groups of cells. In this regard, a wide variety of antibodies specific for individual cell types are known in the art and could be used in conjunction with the receptor to constrain TGF-β activity.

The ability to direct TGF-β into cells which are deficient in their ability to bind and respond to the growth modulatory activities of TGF-β may be desirable for the control of cell growth disorders. In this regard, type III TGF-β receptors conjugated to antibodies having determinants on cells for which therapeutic growth control is desired may be used to direct TGF-β into the cell. Such TGF-β:TGF-β receptor:antibody complexes may be particularly useful in the inhibition of growth in cancer cells that express few if any TGF-β receptors or have somehow lost the ability to respond to the growth-restraining actions of TGF-β.

Knowledge of the type III TGF-β receptor structure may be useful in designing synthetic TGF-β analogues which are capable of inducing the biological effects of the TGF-βs. Moreover, knowledge of TGF-β receptor biology may contribute significantly to understanding the normal growth regulation process, how defects in the process result in unrestrained proliferation in neoplastic cells, and what actions may correct or override these defects and restore normal growth regulatory mechanisms.

Effective methods for controlling the effects of TGF-β on cells expressing TGF-β receptors may include obstructing access to the functional domain of the receptor with receptor-agonists or neutralizing antibodies so that signal induction of TGF-β via the ligand receptor complex is prevented. In this way, the natural ligand, TGF-β, would find fewer receptors available for binding resulting in mitigated signal induction.

Purified type III TGF-β receptors may also find use in the isolation of the TGF-βs and other novel growth factors related to the TGF-β family.

TGF-β receptor was purified by affinity chromatography. Specifically, recombinant TGF-β1 was immobilized onto polymeric resin and used to isolate receptors from a solution containing solubilized HEPM cell membranes as described in Section 7, infra. Recombinant TGF-β1 was obtained in accordance with the method disclosed in United States Patent Application Ser. No. 189,984, which is incorporated by reference herein in its entirety. The invention is not limited to the use of TGF-β1 in the affinity purification of type III TGF-β receptors as TGF-β2, which also binds HEPM cell surface receptors for TGF-β, may also be used. Likewise, other ligands or antibodies capable of binding TGF-β receptor may be employed in conjunction with affinity chromatography techniques to isolate the TGF-β receptor of the invention. Affinity purified receptors may be assayed for their ability to bind ligand as described in Section 7.2., infra.

Once the purified TGF-β receptor of the invention has been obtained, techniques known in the art for determining all or part of its primary structure may be used so that oligonucleotide probes corresponding to specific regions of the amino acid sequence may be designed, constructed and employed in the molecular cloning of TGF-β receptor gene.

5.3. PRODUCTION OF THE TYPE III TGF-β RECEPTOR

The type III TGF-β receptor of the invention may be produced in large quantities by various methods known in the art including but not limited to isolation from large scale culture or fermentation of HEPM cells, other cells naturally expressing the receptor, or recombinant host organisms which direct the synthesis, expression and processing of biologically active type III TGF-β receptor. Alternatively, the type III TGF-β receptor may be chemically synthesized in whole or in part by methods well known in the art, including the solid phase peptide synthesis technique. Purification of homogeneous type III TGF-β receptor may be accomplished by employing the methods described in Section 7, infra, or by other methods known in the art for the purification of proteins.

5.4. MOLECULAR CLONING AND EXPRESSION OF TYPE III TGF-β RECEPTOR GENE

In the practice of the method of the invention, the nucleotide coding sequence for type III TGF-β receptor, or its functional equivalent, can be used to generate recombinant molecules which will direct the expression of the type III TGF-β receptor product. The nucleotide coding sequence for the receptor may be obtained from cells expressing type III TGF-β receptors. For example, the HEPM cell line may be used as the source of the nucleotide coding sequence. The coding sequence may be obtained by cDNA cloning of RNA isolated and purified from such cellular sources or by genomic cloning. Either cDNA or genomic libraries of clones may be prepared from the DNA fragments generated using techniques known in the art, including but not limited to the use of restriction enzymes.

The fragments which contain the gene for type III TGF-β receptor may be identified in a number of ways known in the art. For example, a portion of the receptor amino acid sequence can be used to deduce the DNA sequence, which DNA sequence can then be chemically synthesized, radioactively labeled, and used as a hybridization probe.

Other methods which can be used to isolate the type III TGF-β receptor gene include but are not limited to chemically synthesizing the gene sequence itself from a known sequence which may, for example, be derived from the amino acid sequence of receptor. Alternatively, in vitro translation of selected mRNA followed by functional or immunological assays of the translation products can be used. The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc.

In a particular embodiment, the type III TGF-β receptor gene may may be cloned by taking advantage of the inducible down-regulation of receptors in HEPM source cells. In this regard, applicants have determined that significant down-regulation of the type III TGF-β receptors on HEPM cells can be achieved by treating these cells with TGF-β for 18 hours (Section 6.3.1., infra). It is possible that this observed down-regulation is a reflection of a corresponding reduction in the synthesis of fully processed receptor mRNA.

Similarly, the vaccinia 7.5K promoter may be used.

An alternative expression system which could be used to express receptor is an insect system. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The receptor coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the receptor coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers, (e.g. zinc and cadmium ions for metallothionein promoters). Therefore, expression of the genetically engineered receptor may be controlled. This is important if the protein product of the cloned foreign gene is lethal to host cells. Furthermore, modifications (e.g. glycosylation) and processing (e.g., cleavage) of protein products are important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modificatin of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

5.4.2. IDENTIFICATION OF TRANSFECTANTS OR TRANSFORMANTS EXPRESSING THE TYPE III TGF-β RECEPTOR GENE PRODUCT

The host cells which contain the recombinant type III TGF-β receptor coding sequence and which express the biologically active, mature product may be identified by at least four general approaches: (a) DNA-DNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of receptor mRNA transcripts in the host cell; and (d) detection of the mature gene product as measured by ligand binding ability, immunoassay and, ultimately, by its biological activity.

In the first approach, the presence of the receptor coding sequence inserted in the expression vector can be detected by DNA-DNA hybridization using probes comprising nucleotide sequences that are homologous to the receptor coding sequence.

In the second approach, the recombinant expression vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the receptor coding sequence is inserted within a marker gene sequence of the vector, recombinants containing the receptor coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with the receptor sequence under the control of the same or different promoter used to control the expression of the receptor coding sequence. Expression of the marker in response to induction or selection indicates expression of the receptor coding sequence.

In the third approach, transcriptional activity for the receptor coding region can be assessed by hybridization assays. For example, polyadenylated RNA can be isolated and analyzed by Northern blot using a probe homologous to the receptor coding sequence or particular portions thereof. Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the mature protein product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmunoprecipitation, enzyme-linked immunoassays and the like. The ultimate test of the success of the expression system, however, involves the detection of the biologically active type III TGF-β receptor gene product. Where the host cell secretes the gene product the cell free media obtained from the cultured transfectant host cell may be assayed for receptor activity. Where the gene product is not secreted, cell lysates may be assayed for such activity. Assays such as the binding assays described herein or the like may be used.

Once a clone that produces high levels of biologically active, mature type III TGF-β receptor is identified, the clone may be expanded and receptor may be purified as described herein or by using techniques well known in the art. Such methods include affinity purification, chromatographic methods including high performance liquid chromatography, and the like.

HEPM cells were grown to confluence (1X10^5 cells/well) in 24-well Costar tissue culture plates. Prior to the binding assay, cells were pre-incubated in serum-free growth medium at 37° C for 4 hours to allow for the dissociation of TGF-β from occupied cells surface receptors. The resulting monolayers were washed 2 times in binding buffer (Dulbecco's MEM + 0.1% BSA + 25 mM HEPES). 250 µl of binding buffer containing 0.5 µg/ml [^{125}I]-iodo-TGF-β1 (specific activity of 100 µCi/µg) together with increasing amounts of unlabeled TGF-β1 were added to duplicate wells. Steady state binding was carried out 4° C for 3 hours. After incubation cells were washed 3 times with cold binding buffer and solubilized in 200 µl of Triton solution (20 mM HEPES, 1% Triton X-100, 10% glycerol, 0.01% BSA, pH 7.4). Radioactivity was determined using a gamma spectrometer. Non-specific binding was determined in the presence of a 1,000-fold molar excess unlabeled TGF-β1.

6.1.5. AFFINITY LABELING OF TGF-β CELL SURFACE RECEPTORS

Confluent monolayers of HEPM cells (1X10^5 cell/well) grown in 24-well tissue culture plates (Costar) were washed twice with binding buffer and incubated with 1.0 mL of the same buffer at 37° C for 1.5 hours to dissociate any TGF-β bound to cell surface receptors. The buffer was discarded and the monolayers were incubated at 4° C for 3 hours with 250 µl of binding buffer containing 50 ng/mL of [^{125}I]-iodo-TGF-β - (specific activity of 1000 µCi/µg) in the presence or absence of 1000 ng/mL or unlabeled TGF-β. The monolayers were then washed three times with ice-cold binding buffer and incubated for 15 minutes at 4° C in the presence of 0.5 mL of binding buffer to which 5 µl of 25 mM DSS (disuccinimidyl suberate, Pierce) in DMSO (dimethyl sulfoxide) were added. At the end of this incubation, the monolayers were washed once with a solution of 10 mM Tris, 1 mM EDTA in PBS pH 7.4 to quench the unreacted DSS. The monolayers were solubilized with 100 µl of 1% v/v Triton X-100, 10 mM Tris, 1 mM EDTA, pH 7.0 for 5 minutes at room temperature and the detergent insoluble material was separated by centrifugation at 12,000 xg. The detergent soluble material was immediately processed or frozen at -70° C.

6.2. BIOLOGICAL RESPONSE MEDIATED BY THE TGF-β RECEPTOR

6.2.1. PROLIFERATIVE RESPONSE

The effect of TGF-β on the grown of HEPM cells was deterined by the growth stimulation assays described in Materials and Methods.

Radiolabeled cells were exposed to increasing amounts of TGF-β1 or TGF-β2. The results are presented in FIG. 1a and FIG. 1b, respectively. Both TGF-β1 and TGF-β2 demonstrated similar potencies in stimulating the proliferation of HEPM cells (ED_{50} of 5pM and 2.3 pM, respectively). The results suggest that the action of TGF-β1 and TGF-β2 on HEPM cells is mediated by a single TGF-β receptor.

The proliferative response of HEPM cells to TGF-β treatment as measured by cell counting also implicates a growth factor/receptor-mediated process. Cultures of HEPM cells treated with 50 pM of TGF-β1 reached a cell density twice that observed in untreated cultures by 96 hours post-treatment.

6.2.2. SYNERGISM OF EGF IN THE TGF-β/TGF-β RECEPTOR SYSTEM

The response of HEPM cells to TGF-β treatment in the presence of EGF was determined by radiolabeling the DNA of dividing cells as described in Materials and Methods. A suboptimal dose of TGF-β2 was analyzed for growth stimulatory activity in the presence of EGF. HEPM cells treated with 13 ng/ml EGF alone resulted in a 21% stimulation and with 0.3 ng/ml TGF-β2 alone resulted in a 150% stimulation. HEPM cells treated with both EGF and TGF-β2 at the same doses gave a 511% stimulation, a potentiated response of 340% over that expected from the sum of the two individual responses. The observed synergy between EGF and TGF-β2 indicates that each growth factor stimulates HEPM proliferation via distinct mechanisms; that is, TGF-β action is not mediated directly through the EGF receptor but through a TGF-β-

The following example describes the affinity purification of the type III TGF-β receptor from HEPM cells. The purified receptor thus obtained was characterized functionally and structurally.

7.1. MATERIALS AND METHODS

7.1.1. PREPARATION AND SOLUBILIZATION OF MEMBRANES

HEPM cells were grown to confluence in tissue culture flasks, T150 (Costar), or roller bottles, 850 cm^2 (Costar), in minimal essential medium (MEM, Gibco, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (BIOCEL), 600 µg/ml L-glutamine, 500 µg/ml penicillin and 500 µg/ml streptomycin in a humidified 5% CO_2, 95% air at 37° C controlled atmosphere, or CO_2 atmosphere at 37° C (warm room, roller bottles) rotating at 5-7 revolutions per minute. The confluent monolayers were washed twice with PBS and incubated for 48 hours in fresh growth media without fetal bovine serum in the above-described growth conditions. The monolayers were again washed with ice-cold PBS and the cells were solubilized for 5 minutes at room teperature with a solution containing 1% Triton X-100, 25 mM Hepes pH 7.4. The detergent soluble material was separated from the insoluble material by centrifugation at 12,000 xg. The detergent soluble material was collected and processed immediately or frozen at -70° C.

7.1.2. IMMOBILIZATION OF TGF-β TO SOLID SUPPORT

TGF-β samples purified to homogeneity by reverse phase HPLC (Gentry, et al., 1988, Mol. Cell. Biol. 8:4162) were reduced to half the original volume by vacuum under centrifugation (SpeedVac) to eliminate the acetonitrile. The volume was determined and the solution was adjusted to 0.1 M sodium phosphate (using a 1:10 dilution of 1M sodium phosphate stock pH 7.5), 100 mM NaCl (using a stock of 1 M NaCl). One milliliter Affi-Gel 10 resin (BioRad) previously washed with ice cold H_2O was incubated with the adjusted TGF-β solution at 4° C overnight with end-over-end mixing. The resin was extensively washed with a solution containing 1% Triton X-100, 25 mM Hepes pH 7.4, 100 mM NaCl, and stored at 4° C.

About 2 ml of HPLC-purified recombinant TGF-β1 at a concentration of 250 µg/ml was coupled to 1 ml of packed Affi-Gel-10 beads using this procedure.

7.1.3. AFFINITY LABELING OF SOLUBLE TGF-β RECEPTORS

Solubilized receptor samples (50 µl) were diluted with three volumes of binding buffer containing 0.5% v/v Triton X-100 and 50 ng/ml [^{125}I]-iodo-TGF-β. The samples were incubated at 4° C for 3 hours in the presence or absence of 1,000 ng/ml unlabeled TGF-β. Four microliters (4 µl) of 10 mM DSS in DMSO were then added. After 15 minutes the unreacted DSS was quenched by adding 20 µl of 100 mM Tris/HCl pH 7.0.

Affinity-labeled samples were incubated at 4° C for 2 hours with 15 µl of packed WGA-sepharose beads (wheat germ aglutinin-sepharose, Sigma) with end-over-end mixing. The beads were sedimented by centrifugation at 12,000 xg for 1 minute and washed three times with a buffer solution containing 0.5% v/v Triton X-100, 10 mM Tris/HCl, 1 mM EDTA. The washed, packed beads were heated for 5 minutes at 95° C in the presence of 20 µl of electrophoresis sample buffer containing 5% 2-mercaptoethanol. The supernatants were retained for SDS-PAGE analysis.

7.1.4. SDS-PAGE AND AUTORADIOGRAPHY

TABLE I

AMINO ACID COMPOSITION OF AFFINITY PURIFIED TYPE III TGF-β RECEPTOR[1]					
Amino Acid[2]	pMoles A. A.	Mole % M/100M	Residues nG A.A. Per Mole	Residues Per Mole	Integer Res/Mol
ASx	266.00	11.55	12424	107.96	108
GLx	160.00	6.95	8384	64.94	65
SER	165.00	7.16	5831	66.96	67
GLY	279.00	12.11	6460	113.23	113
HIS	54.00	2.34	3006	21.92	22
ARG	155.00	6.73	9825	62.91	63
THR	91.00	3.95	3734	36.93	37
ALA	240.00	10.42	6923	97.40	97
PRO	112.00	4.86	4414	45.45	45
TYR	87.00	3.78	5762	35.31	35
VAL	146.00	6.34	5874	59.25	59
MET	0.00	0.00	0	0.00	0
ILE	77.00	3.34	3536	31.25	31
LEU	221.00	9.60	10150	89.69	90
PHE	87.00	3.78	5197	35.31	35
LYS	163.00	7.08	8479	66.15	66
TOTAL	2303.00	100.00	100000	934.66	933

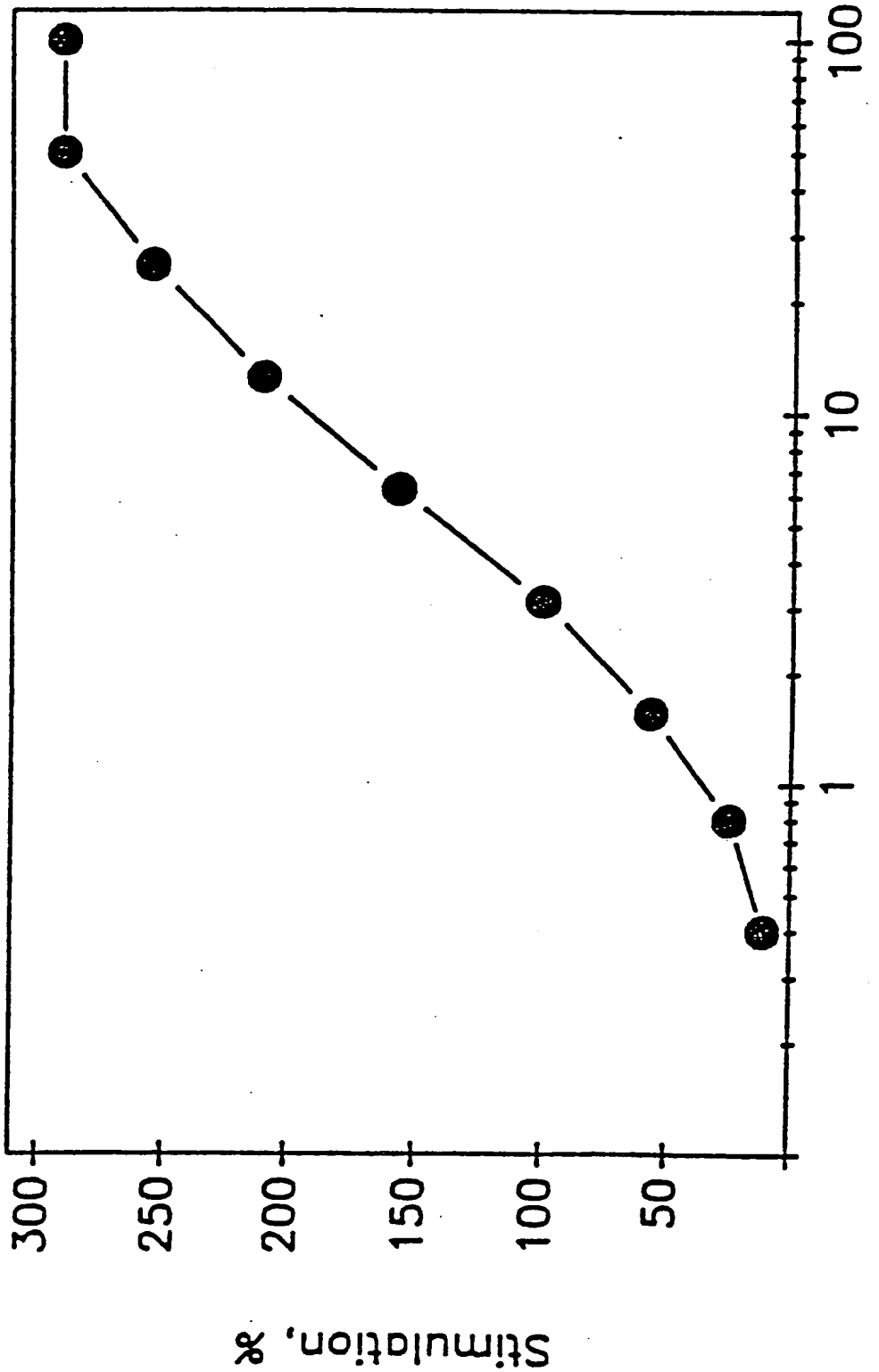
[1] A molecular weight assumption of 100 Kd was used in these calculations.
[2] Cystein and tryptophan residues not determined

The first 7 amino-terminal amino acids of the type III TGF-β receptor were determined through analysis performed in a automated gas phase sequencer (ABI model 470). The assigned amino acids are: NH_2-Lys-Tyr-Tyr-Asp-Lys-Asp-Tyr

Claims

1. A purified protein that:
   (a) has an amino-terminal amino acid sequence comprising:
NH_2-Lys-Tyr-Tyr-Asp-Lys-Asp-Tyr
   (b) has a molecular weight of about 100,000 to about 220,000 daltons
   (c) is a receptor for TGF-β
2. A glycosylated protein of claim 1.
3. An un-glycosylated protein of claim 1.
4. The protein of claim 1 or a peptide or fragment thereof that specifically binds TGF-β.
5. The protein of claim 2 in which the TGF-β is natural TGF-β1.
6. The protein of claim 2 in which the TGF-β is recombinant TGF-β1.
7. The protein of claim 2 in which the TGF-β is natural TGF-β2.
8. The protein of claim 2 in which the TGF-β is recombinant TGF-β2.
9. The protein of claim 6 having a dissociation constant of about 1×10^{-12}M to about 1×10^{-9}M.
10. Purified type III TGF-β receptor, obtained by the method comprising:
   (a) solubilizing membranes from cultured HEPM cells
   (b) affinity purifying type III TGF-β receptor there from using TGF-β as the ligand.
11. A purified protein that:
   (a) has a molecular weight of about 100,000 to about 220,000 daltons
   (b) specifically binds TGF-β.
12. The protein of claim 11 in which the TGF-β is natural TGF-β1.
13. The protein of claim 11 in which the TGF-β is recombinant TGF-β1.
14. The protein of claim 11 in which the TGF-β is natural TGF-β2.

FIG. 1A



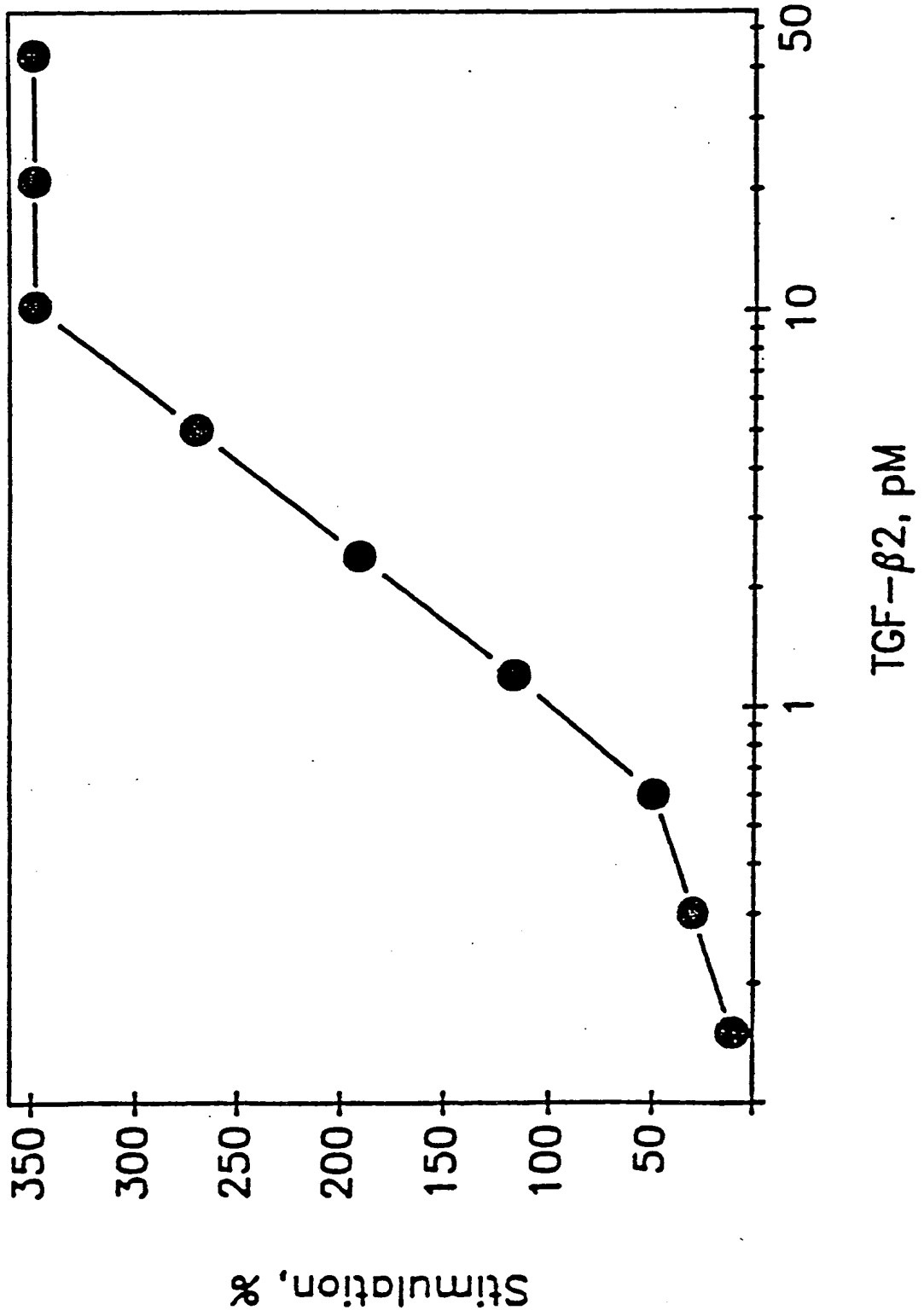


FIG. 1B

FIG. 2

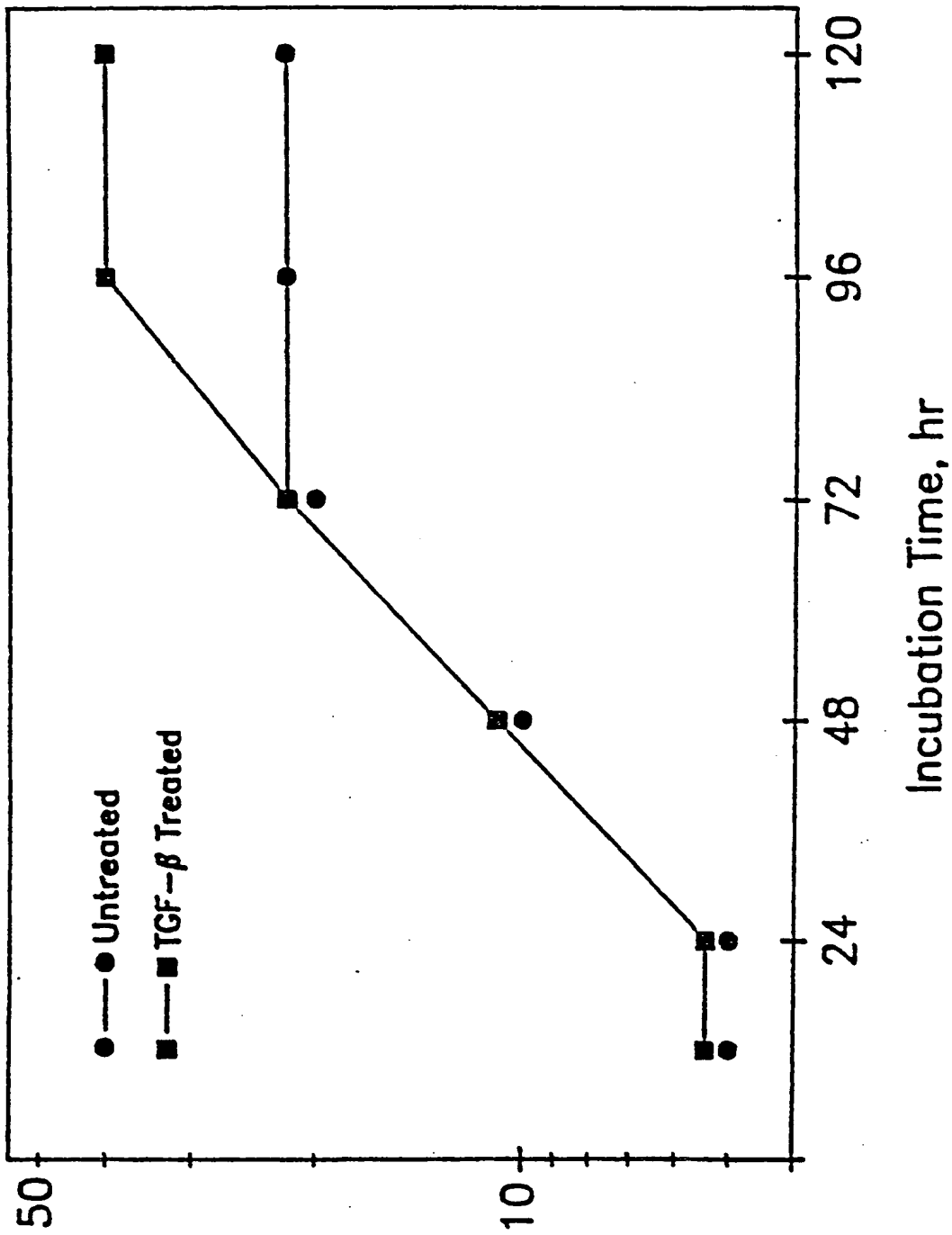


FIG. 3A

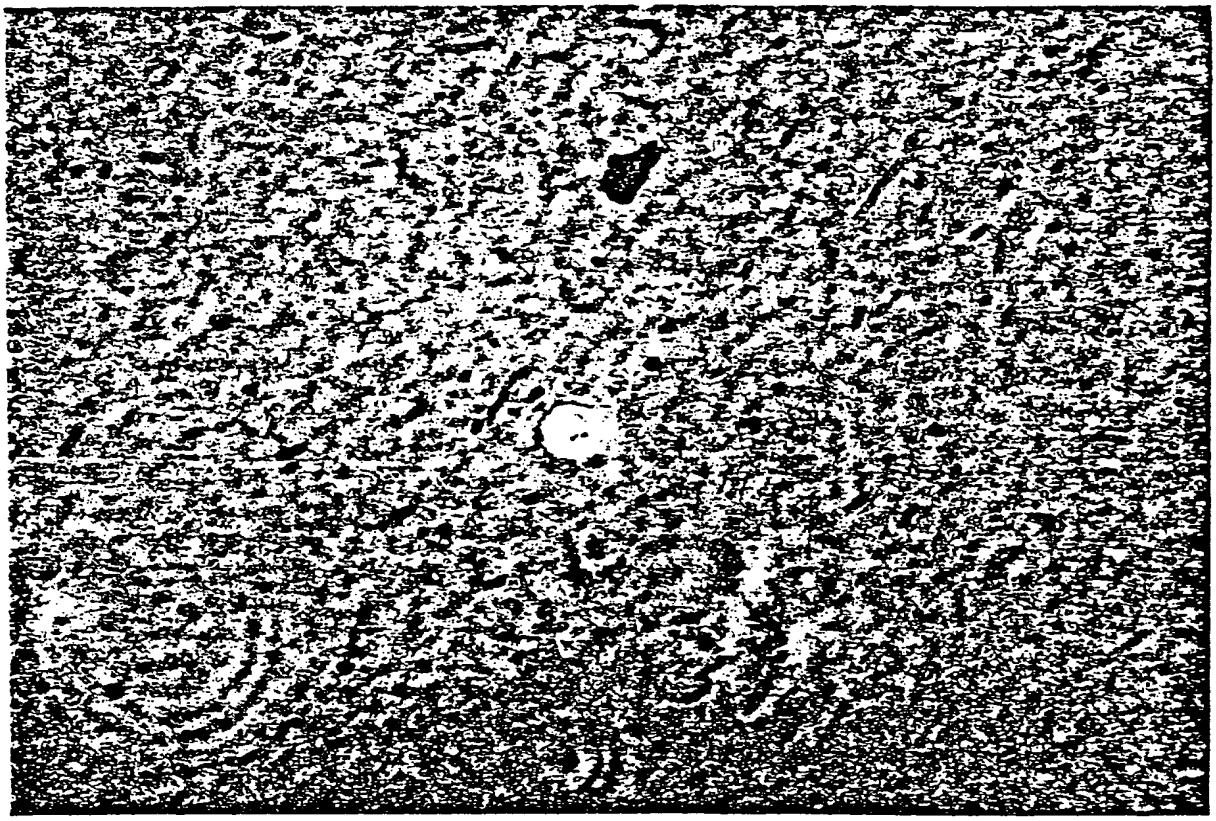


FIG. 3B

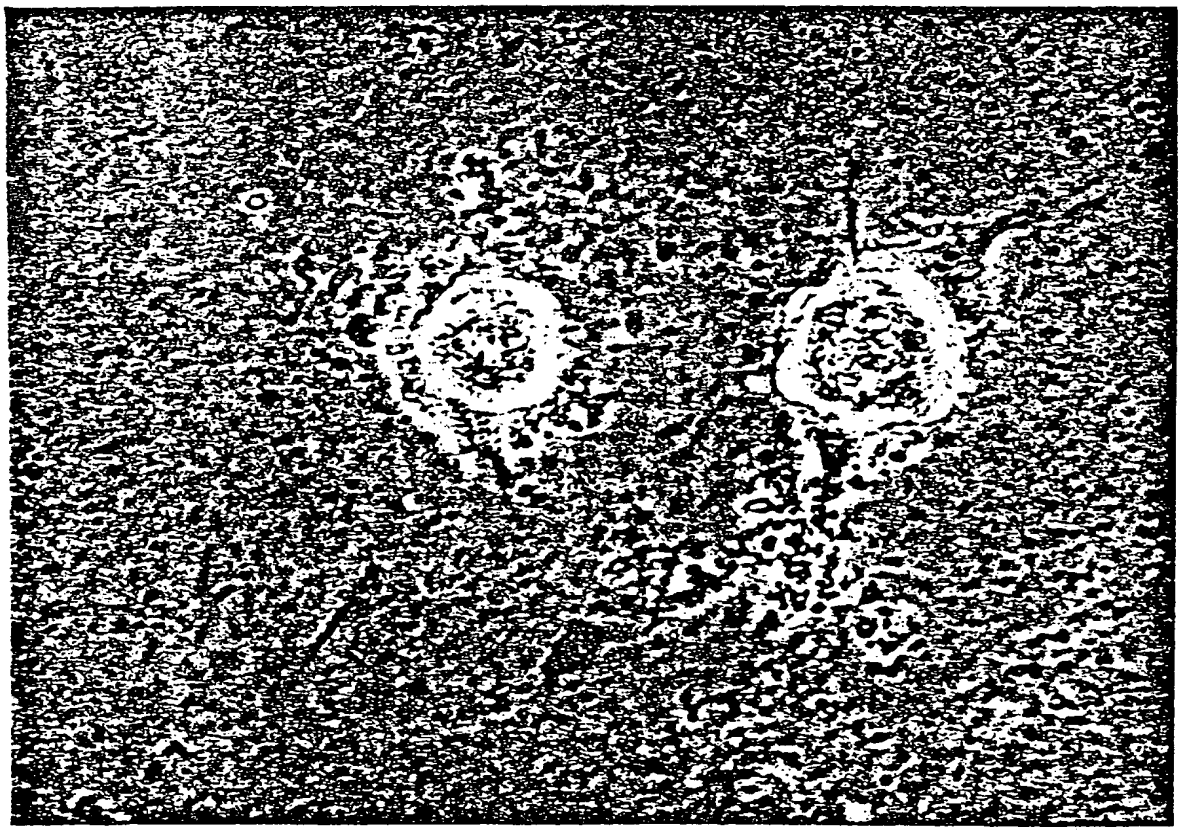
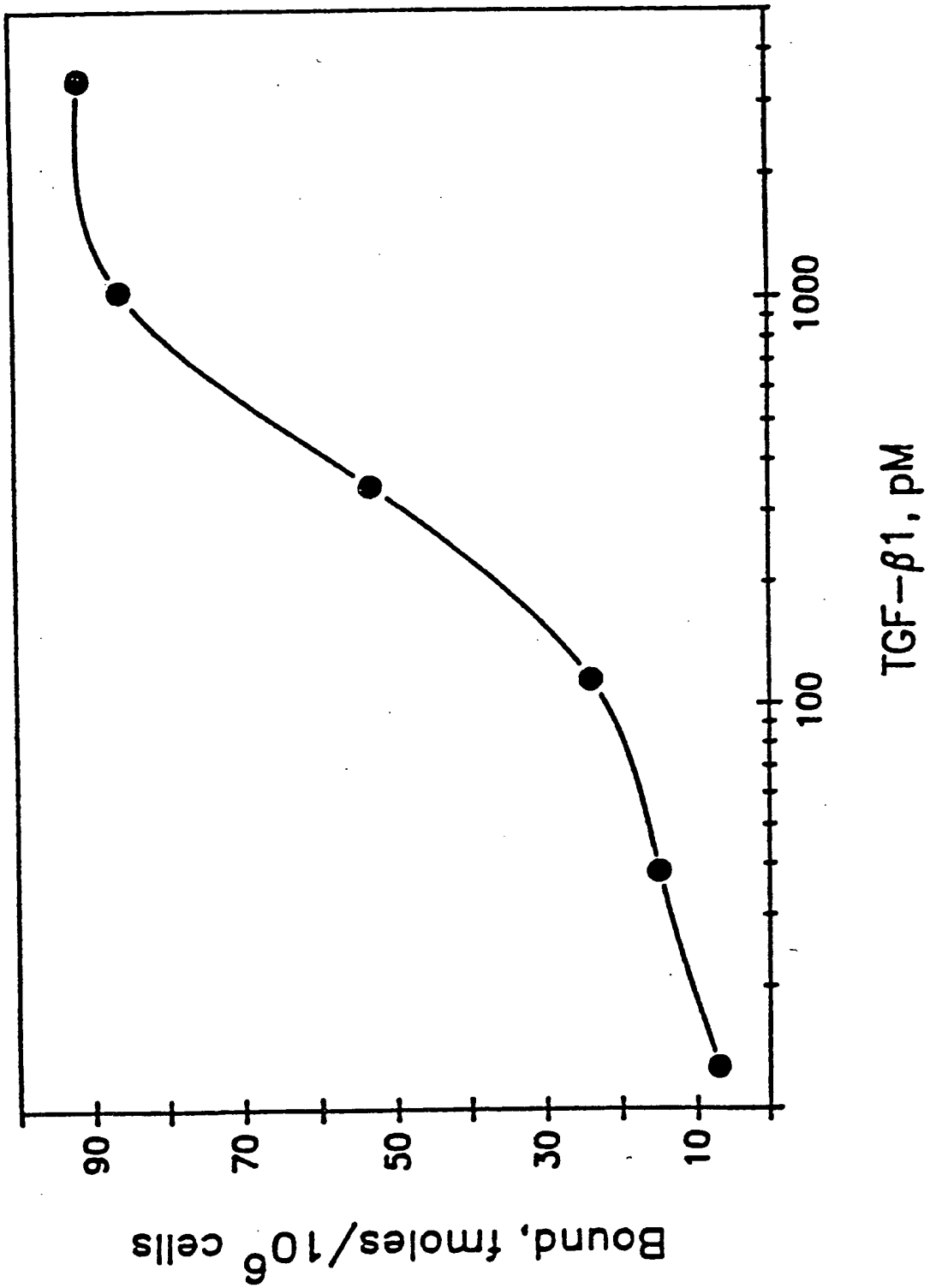


FIG. 4A



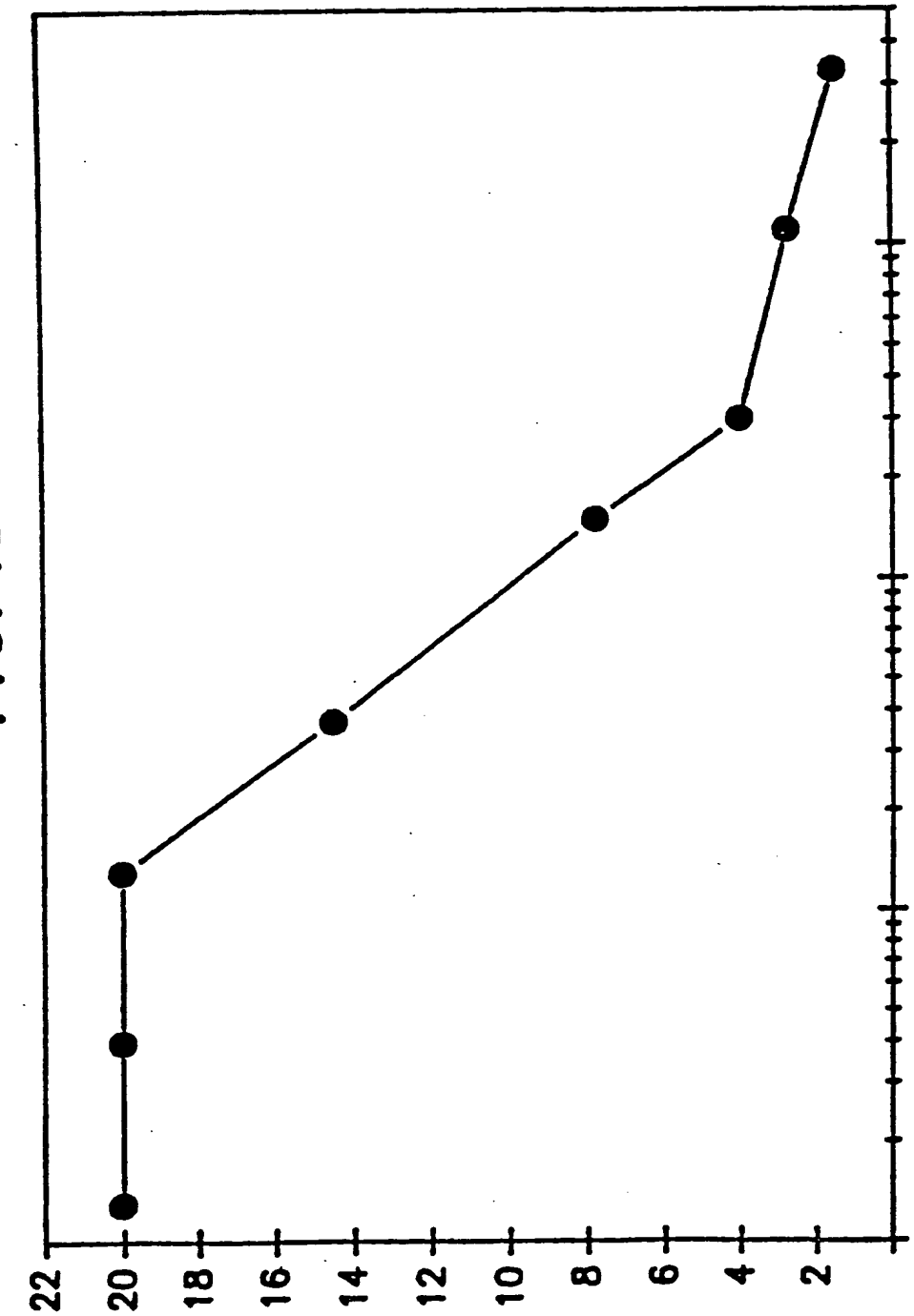


FIG. 4B

FIG. 5

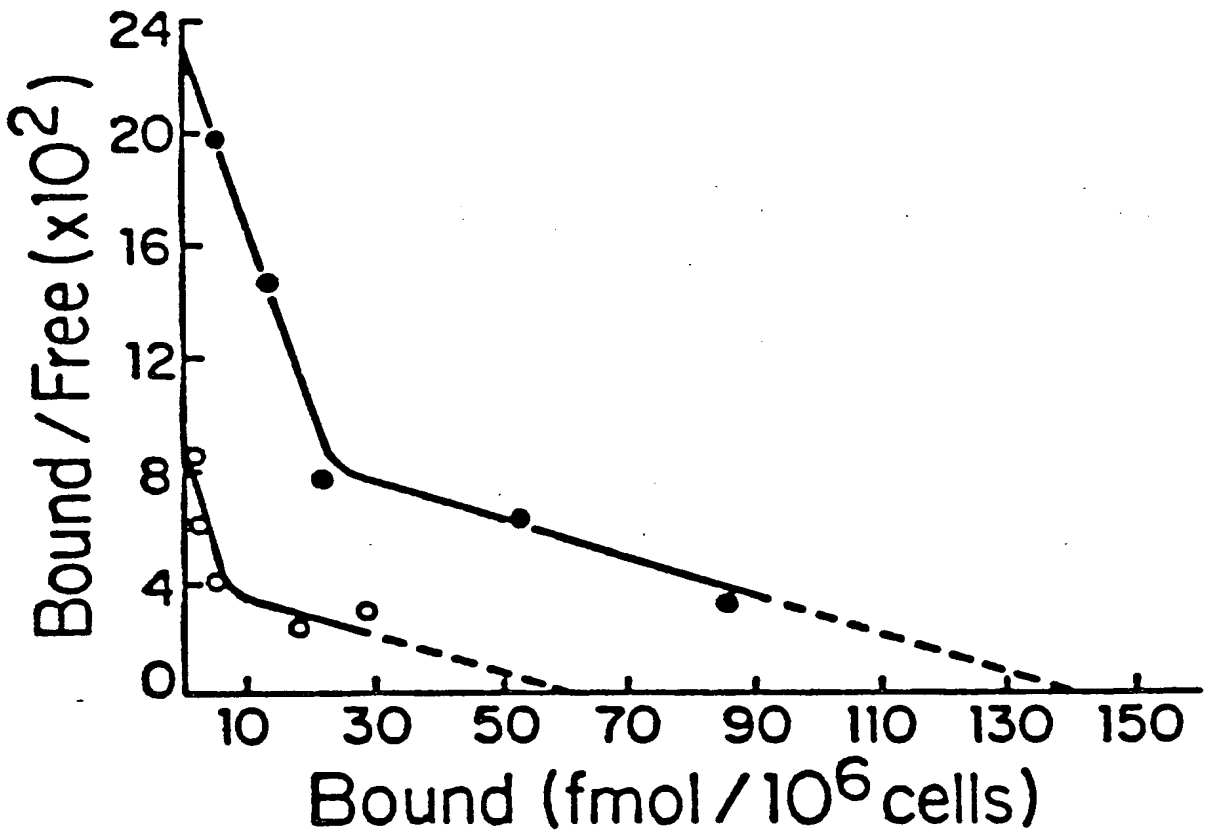


FIG. 6

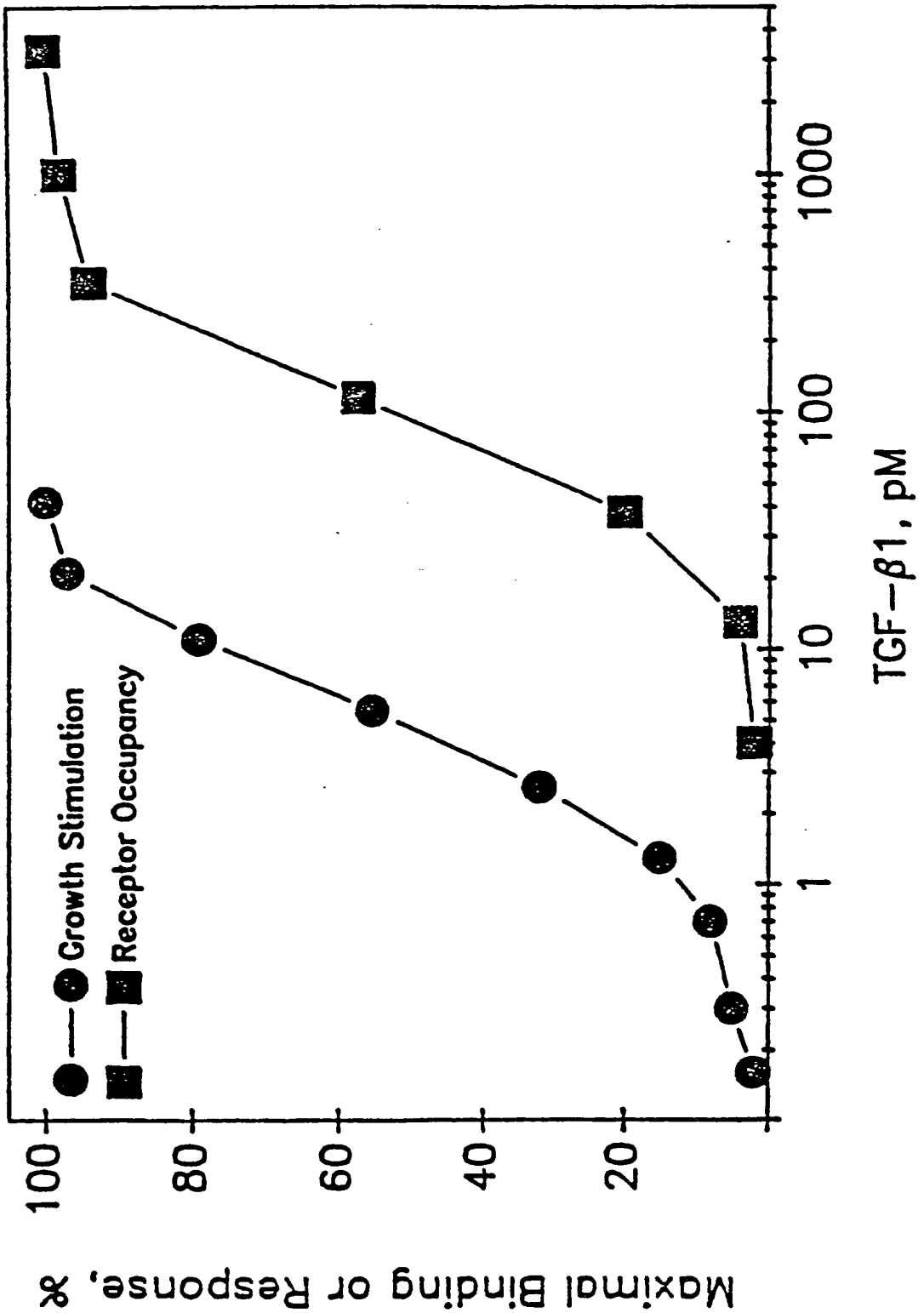


FIG. 7

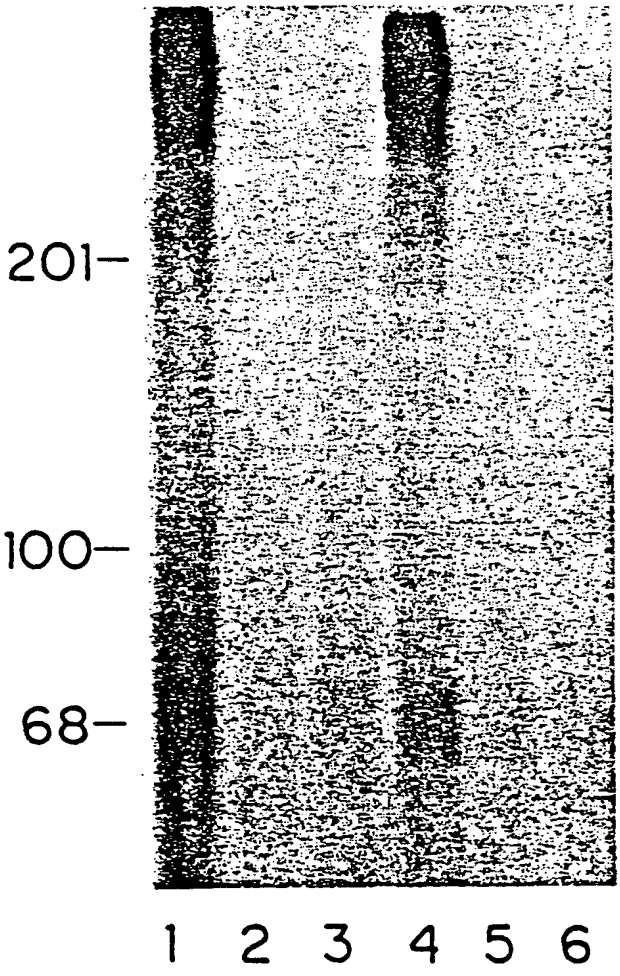


FIG. 8

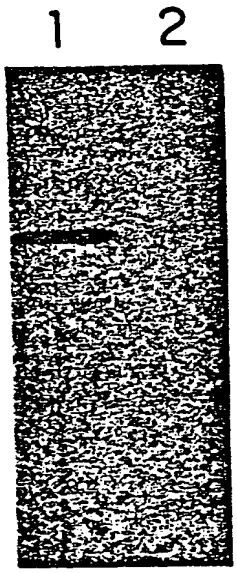


FIG. 9

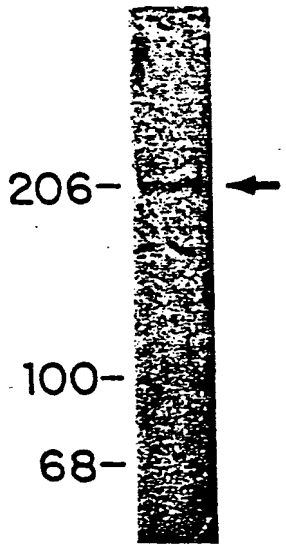
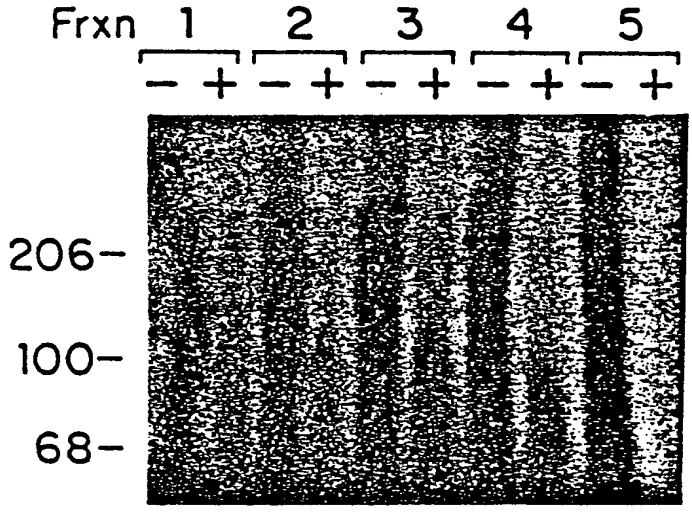


FIG. 10



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EUROPEAN SEARCH REPORT

Application Number

EP 89 40 3084

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	METHODS IN ENZYMOLOGY, vol. 146, 1987, pages 174-195, PEPTIDE GROWTH FACTORS, PART A, ed. Barnes et al., Academic Press, Orlando, US; J. MASSAGUE: "Identification of receptors for type-beta transforming growth factor" * Pages 174-195, esp. pages 182-195 *	11-15	C 07 K 15/12 C 07 K 15/14 C 12 N 15/00 C 07 K 3/20
X	BIOCHEMISTRY, vol. 25, no. 11, 1986, pages 3083-3091, Washington, US; B.O. FANGER et al.: "Structure and properties of the cellular receptor for transforming growth factor type beta" * Pages 3085-3091 (Results & discussion) *	11-15	
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 261, no. 21, 25th July 1986, pages 9972-9978, Baltimore, US; S. CHEIFETZ et al.: "Cellular distribution of type I and type II receptors for transforming growth factor-beta" * Pages 9973-9976 (Results) *	11-15	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)
C 07 K C 12 N

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	21 June 91	KORSNER S.E.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
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C 12 N

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